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Multicenter Clinical Evaluation of the *illumi*gene Group A *Streptococcus* DNA Amplification Assay for Detection of Group A *Streptococcus* from Pharyngeal Swabs

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Acute pharyngitis is a nonspecific symptom that can result from a number of viral or bacterial infections. For most etiologies, symptoms are self-limited and resolve without lasting effects; however, pharyngitis resulting from infection with *Streptococcus pyogenes* (a group A *Streptococcus* [GAS]) can be associated with serious sequelae, including acute rheumatic fever and acute glomerulonephritis. Rapid accurate detection of GAS in pharyngeal specimens from individuals suffering from pharyngitis aids in the management and selection of antibiotic therapy for these patients. A total of 796 pharyngeal swabs were collected at three separate clinical centers. Each specimen was analyzed using the *illumi*gene group A strep DNA amplification assay (Meridian Bioscience Inc., Cincinnati, OH). To confirm GAS identification, the results were compared to those from direct and extracted culture methods using Gram staining and a GAS-specific latex agglutination test. Discrepant results were resolved using an alternative nucleic acid amplification test. The prevalence of culture-detected GAS in this study was 12.8% (102/796 specimens). The *illumi*gene assay detected GAS in 74/74 direct culture-positive specimens (100% sensitivity) and 100/102 extracted culture-positive specimens (98.0% sensitivity). GAS was detected by the *illumi*gene assay in an additional 42 specimens that were direct culture negative (94.2% specificity) and 16 specimens that were extracted culture negative (97.7% specificity). Discrepant analysis using an alternative molecular assay detected GAS nucleic acid in 13/16 (81.3%) false-positive specimens and 1/2 false-negative specimens, resulting in a final sensitivity of 99.0% and a specificity of 99.6% for the detection of GAS in pharyngeal swabs using the *illumi*gene assay.

Group A *Streptococcus* (GAS) is a commonly encountered pathogen associated with a variety of diseases, from minor wound infections to life-threatening toxic shock syndrome. It is the most common bacterial cause of pharyngitis, accounting for 10 to 30% of all cases (1–3). While GAS has the potential to cause acute pharyngitis in all age groups, the highest prevalence is seen in children between 5 and 12 years of age (4) and might be linked to crowding within schools. In a study by Pfoh et al. (5), the cost per case of GAS pharyngitis was estimated to be approximately $205, with about half of the costs attributed to nonmedical costs such as missed days of work by parents for child care.

Clinical manifestations of GAS and viral pharyngitis are similar, making diagnosis on the basis of physical examination difficult. Patients present with symptoms of severe pharyngitis but also might exhibit systemic symptoms, including fever, malaise, abdominal pain, and headache. Physical examination also might reveal lymphadenopathy or tonsillar exudates. GAS pharyngitis is usually self-limited and resolves without the need for antibiotic treatment (6); however, a minority of patients develop severe complications such as scarlet fever and peritonsillar cellulitis, as well as immune-mediated complications, including poststreptococcal glomerulonephritis and acute rheumatic fever. As a result, diagnostic algorithms based on clinical symptoms, physical examination results, and epidemiology have been designed to identify patients who likely have GAS pharyngitis, as opposed to viral pharyngitis (7). Unfortunately, even when correctly applied by experienced physicians, this method has been shown to have a positive predictive value of only 35% to 50% for the diagnosis of GAS pharyngitis, due to the broad overlap in symptoms between the viral and bacterial etiologies (8). As a result, current Infectious Diseases Society of America (IDSA) guidelines state that a clinical diagnosis of GAS pharyngitis must be confirmed (8).

The current gold standard for laboratory diagnosis of GAS pharyngitis is culture of pharyngeal swab specimens (9). Cultures are screened for the presence of beta-hemolytic colonies, which are positively identified as GAS using standard biochemical tests (e.g., catalase, pyrrolidonyl arylamidase, and latex agglutination for type-specific antigen tests) (1). While sensitive, culture requires up to 48 h of incubation, which is problematic for physicians considering antibiotic therapy as an option for treating acute pharyngitis.

Alternative methods to speed the time to diagnosis of GAS pharyngitis include point-of-care (POC) antigen detection tests and nucleic acid-based methodologies. POC rapid antigen tests detect the presence of the group A carbohydrate and do not require culture. Rapid antigen assays are advantageous because they provide results within minutes, enabling physicians to make prospective decisions about treatment (10). These tests are specific but exhibit only 72% to 90% sensitivity compared to that of culture (11). Molecular tests for the detection of GAS have been described, and the sensitivities of these tests range from 88.6% to

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TABLE 1 Performance of the *illumi*gene group A *Streptococcus* assay compared to that of the standard culture

Clinical test site no.	No. of specimens tested	Results (no.)[a]				Performance (% [95% CI])[b]			
		TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV
1	338	40	11	287	0	100 (91–100)	96.3 (93–98)	78.4 (64–88)	100 (98–100)
2	241	18	18	205	0	100 (81–100)	91.9 (87–95)	50 (32–67)	100 (98–100)
3	217	16	13	188	0	100 (79–100)	93.5 (89–96)	55.2 (35–73)	100 (98–100)
Total	796	74	42	680	0	100 (95–100)	94.2 (92–95)	63.8 (54–72)	100 (99–100)

[a] TP, true positive; FP, false positive; TN, true negative; FN, false negative.
[b] CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

93.0% with >97% specificity (12, 13); however, currently only one such test has been cleared by the U.S. Food and Drug Administration (FDA).

In this study, we evaluated the recently FDA-cleared *illumi*gene group A *Streptococcus* DNA amplification assay (Meridian Bioscience Inc., Cincinnati, OH) for the detection of GAS in pharyngeal swab specimens. The results are compared to those of routine culture using Lancefield antigen agglutination typing as the gold standard for the identification of GAS.

MATERIALS AND METHODS

Specimen enrollment and reference culture. A total of 796 dual pharyngeal swabs from patients ranging in age from <1 to 87 years were collected in liquid Stuart (LS) transport medium (BD, Sparks, MD). Swabs were collected at three geographically distinct clinical centers and were included in this study in accordance with site-specific institutional review board-approved protocols. Reference cultures were conducted by inoculating each swab specimen on a Trypticase soy agar plate with 5% sheep blood (blood agar plate [BAP]). Inoculated BAPs were incubated at 35°C in an aerobic atmosphere for 48 h. Following incubation, cultures were examined for the presence of small beta-hemolytic colonies characteristic of GAS. Identification was confirmed using Gram staining (Gram-positive cocci in chains), the catalase test (nonreactive), and a GAS-specific latex agglutination test (the PathoDx strep grouping kit [Thermo Fisher Scientific, Waltham, MA] or the Streptocard acid latex group kit [BD, Sparks, MD]). To maximize culture sensitivity, following direct plating of the specimen, approximately 50 μl of transport medium was extracted from each residual LS pledget, plated on a BAP, and incubated at 35°C for 48 h (extracted culture method).

***illumi*gene group A *Streptococcus* test.** To analyze swab specimens using the *illumi*gene group A *Streptococcus* DNA amplification assay (Meridian Bioscience Inc.), swab tips were broken off into sample preparation tubes. These tubes were vortexed for 10 seconds. Following vortexing, 10 drops of the specimen were transferred to a heat treatment tube and incubated at 95°C for 10 min. Following heat treatment, 50 μl of lysate was transferred to both the *illumi*gene test and *illumi*gene control chambers. The test device was then inserted into the *illumi*pro-10 incubator/reader, and amplification was initiated. This assay uses loop-mediated amplification (LAMP) technology to target a highly conserved 206-bp sequence of the *Streptococcus pyogenes* pyrogenic exotoxin (*speB*) gene. Nucleic acid amplification is detected indirectly by the presence of precipitated pyrophosphate, which is elaborated during elongation of the DNA template. The presence of turbidity (precipitated magnesium pyrophosphate) within the test device was read by the *illumi*pro-10 device, and the results were available within 40 min.

Analysis of discrepant results. Cultures that gave discordant results between the *illumi*gene GAS test and the extracted culture method were analyzed using a laboratory-developed molecular test employing TaqMan probes and a Rotor-Gene real-time PCR thermocycler (Qiagen, Germantown, MD). The TaqMan probes were directed against a region of the *speB* gene different from that targeted by the *illumi*gene GAS assay.

RESULTS

Prospectively collected specimens with the routine culture method. The performance of the *illumi*gene group A *Streptococcus* assay was evaluated on 796 deidentified remnant throat swabs by using the routine culture method as the gold standard comparator (Table 1). A total of 680 (85.4%) specimens tested negative with both the *illumi*gene assay and standard culture. Of the 116 specimens that tested positive by the *illumi*gene assay, 74 (63.8%) were also positive by reference culture, while 42 (36.2%) were negative. No culture-positive *illumi*gene-negative (i.e., false-negative) results were observed. The sensitivities were 100% at all three study sites, and the specificities ranged from 91.9% to 96.3%, compared to those for routine culture. This resulted in overall sensitivity and specificity values of 100% (95% confidence interval [CI], 95% to 100%) and 94.2% (95% CI, 92% to 95%), respectively. Although the majority of specimens (86.9%; 692/796) tested were obtained from pediatric patients (age, <19 years), performance results were similar when the adult and pediatric populations were compared.

Prospectively collected specimens with the extracted culture method. To increase the sensitivity of the culture approach, extracted culture also was performed on each specimen (see Materials and Methods). Extracted culture results were compared to those obtained from the *illumi*gene group A *Streptococcus* assay (Table 2). A total of 678 specimens (85.2%) tested negative by both the *illumi*gene and the enriched culture methods. Of those that tested positive by the *illumi*gene assay ($n = 116$), 100 (86.2%) were positive by enriched culture and 16 (13.8%) were negative. Two samples tested negative by the *illumi*gene assay but were positive by enriched culture (i.e., false negative). Across all three study sites, the sensitivities ranged from 93.3% to 100% and the specificities ranged from 96.2% to 98.6%, with an overall sensitivity of 98% (95% CI, 93% to 99%) and an overall specificity of 97.7% (95% CI, 96% to 98%).

Discrepant analysis. Discrepant analysis was performed using a laboratory-developed PCR assay and was conducted on the 18 cultures that were categorized as either false negative or false positive by the *illumi*gene assay compared to the extracted culture (Table 3). Of the false-positive results, 13/16 specimens (81.3%) were negative by the standard and extracted culture methods but positive by the alternative PCR method. Both specimens that were categorized as false negative were positive by the extracted culture method only. One of the two specimens tested positive using the alternative PCR method, while the other remained negative. Therefore, following discrepant analysis using an alternative molecular method, the final sensitivity and specificity values for the *illumi*gene assay were 99.0% and 99.6%, respectively.

TABLE 2 Performance of the *illumi*gene group A *Streptococcus* assay compared to that of the extracted culture

Clinical test site no.	No.	Result (no.)[a]				Performance (% [95% CI])[b]			
		TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV
1	338	47	4	287	0	100 (92–100)	98.6 (96–99)	92.2 (81–97)	100 (98–100)
2	241	28	8	203	2	93.3 (77–99)	96.2 (92–98)	77.8 (60–89)	99 (96–99)
3	217	25	4	188	0	100 (86–100)	97.9 (94–99)	86.2 (68–96)	100 (98–100)
Total	796	100	16[c]	678	2[d]	98.0 (93–99)	97.7 (96–98)	86.2 (78–91)	99.7 (98–99)

[a] TP, true positive; FP, false positive; TN, true negative; FN, false negative.
[b] CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.
[c] Thirteen of 16 specimens were positive for GAS using an alternative molecular assay.
[d] One of 2 specimens was negative for GAS using an alternative molecular assay.

DISCUSSION

The gold standard for diagnosis of group A streptococcal pharyngitis is 48-h culture on blood agar (1). Unfortunately, the decision to treat a patient with antibiotics is often made before culture results are available. This necessitates the development of rapid and accurate diagnostic approaches. Algorithms utilizing clinical findings have been shown to lack sensitivity and specificity (7). Guidelines published in 2012 by the IDSA recommend against using such algorithms and state that the diagnosis of GAS pharyngitis should be based on objective laboratory results (8).

Currently available POC rapid antigen tests lack sensitivity; further, the prevalence of asymptomatic GAS colonization in children and adolescents is higher than that seen in adults (8). For these reasons, IDSA guidelines dictate that negative rapid antigen detection tests for pediatric and adolescent patients be confirmed by throat culture to enhance the diagnostic sensitivity (8, 14).

Molecular assays have demonstrated sensitivity characteristics superior to those of POC rapid antigen tests without a loss of specificity, making this an attractive option for laboratory diagnosis of GAS. Until now, the only FDA-cleared molecular test was the GASDirect test (Gen-Probe, San Diego, CA). This test exhibits 88.6% sensitivity and 97.8% specificity compared to culture, making it relatively reliable (12). It also allows for batched samples, making large-scale testing possible. While these aspects make it an attractive testing option, it is not without limitations that prevent widespread use. The need for multiple pieces of instrumentation and multiple steps in sample preparation is a significant barrier to adapting this test to a point-of-care setting.

The LightCycler Strep A assay, licensed by Roche, is a molecular test currently in use as a laboratory-developed test in some laboratories. In contrast to the GASDirect test, this assay utilizes PCR-based DNA amplification for the detection of GAS in patient specimens. The application of PCR to the diagnosis of GAS pharyngitis imparts a high level of accuracy to the test, which exhibits a 93% sensitivity and 98% sensitivity compared to those of standard culture methods (13). Additionally, melting curve analysis enables the detection and differentiation of group C and group G *Streptococcus* from GAS in specimens (13). Despite these advantages, a lack of FDA-cleared molecular tests has prevented the widespread use of these tests in clinical laboratories.

Herein, we have evaluated the *illumi*gene group A *Streptococcus* DNA amplification assay in a large multicenter study employing prospectively collected clinical samples. This test uses loop-mediated amplification (LAMP) technology in a process by which designed primers allow specific isothermal amplification of DNA. A by-product of the reaction, magnesium pyrophosphate, forms a white precipitate, which is measured as turbidity. The presence of turbidity indicates DNA amplification and the presence of the primer target, whereas the absence of turbidity indicates the absence of the primer target. This assay is performed in a test chamber using group A *Streptococcus*-specific primers and also is performed in parallel in a control chamber using *Staphylococcus aureus*-specific primers and *S. aureus* DNA (internal control).

Data from this study establish the high sensitivity of this assay, i.e., 100% (95% CI, 95% to 100%) sensitivity in comparison with that of the standard culture and 98% (95% CI, 93% to 99%) sensitivity in comparison with that of the enriched culture. Only two specimens (0.25%) reported as negative with the *illumi*gene test were found to be positive by enriched culture. Both were negative by routine culture, suggesting that the failure of the *illumi*gene assay to detect GAS in these samples was the result of low concentrations of organisms in the samples. Of note, one of these specimens was negative by alternative PCR as well. This might indicate the presence of a possible inhibitor of molecular testing within the sample itself.

Of the 16 specimens with apparent false-positive results with the *illumi*gene assay, 13 were also positive by an alternative PCR method. These 13 samples might represent the presence of GAS nucleic acid in the absence of viable organisms and might have come from patients who had resolving infections or already were

TABLE 3 Discrepant analysis of the *illumi*gene group A *Streptococcus* assay compared to standard culture and enriched culture

No. of samples	*illumi*gene assay result	Standard culture		Enriched culture		PCR result
		Result	Result type[a]	Result	Result type	
3	Positive	Negative	FP	Negative	FP	Negative
13	Positive	Negative	FP	Negative	FP	Positive
1	Negative	Negative	TN	Positive	FN	Not tested
1	Negative	Negative	TN	Positive	FN	Negative

[a] FP, false positive; TP, true negative; FN, false negative.

undergoing antimicrobial therapy. Importantly, current POC rapid antigen tests also do not differentiate between viable and nonviable organisms (11, 12).

In addition to high sensitivity and specificity, a significant advantage to molecular methods is their potential utility as rapid diagnostic tests. Results are available within 1 h of setup, enabling physicians to initiate appropriate treatment promptly. Alternatively, since tests can be batched easily, testing can be performed several times a day in laboratories with high throughput. Testing can potentially be coupled with a method of automated physician notification, as described by Uhl et al. (13). This strategy would offer results with more rapidity than the standard 48-h culture.

It is important to acknowledge the limitations of the *illumi*gene group A *Streptococcus* assay which preclude its use as a sole means for diagnosing pharyngitis. While the assay is very sensitive, it identifies only pharyngitis caused by GAS. Culture is still needed to detect other causes of pharyngitis (i.e., group C *Streptococcus*, group G *Streptococcus*, and viral etiologies). Although not as prevalent as GAS, these non-GAS streptococci are responsible for up to 5% of the total number of cases of pediatric pharyngitis (1). Further, because this assay is only qualitative, it is unable to distinguish between active infection and low-level or asymptomatic carriage of GAS as part of the normal pharyngeal flora. This is also a limitation of antigen-based tests. Pharyngeal carriage rates have been reported to be as high as 33% in some regions (15). Positive results for such patients might not warrant antibiotic administration.

Three strengths of this study were (i) the number of enrolled samples ($n = 796$), (ii) the multicenter nature of the study (three separate sites), and (iii) the comparisons with a standard culture, an enriched culture, and an alternative PCR test. Routine nonenriched culture is the gold standard for detection, though comparison to more sensitive culture and PCR methods enabled a more complete representation of the performance of the *illumi*gene test.

Recent IDSA guidelines recommend that the diagnosis of GAS pharyngitis be made on the basis of laboratory results rather than clinical algorithms (8). This is a challenge, given the poor sensitivity of currently available rapid tests. In fact, improvement of rapid tests for the detection of GAS pharyngitis was specifically identified as an area in need of further research. The *illumi*gene group A *Streptococcus* assay is a rapid accurate test with high sensitivity and specificity for the detection of GAS. It is easy to perform and provides reproducible results among different users in different settings, making it applicable to a variety of clinical environments. These aspects make it a useful diagnostic tool, an attractive alternative to other rapid diagnostic tests for GAS pharyngitis, and an answer to the challenge of rapid test improvement put forth by recent IDSA guidelines.

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